AMENDMENTS TO THE SPECIFICATION

Please amend paragraph [00110] as follows:

For many applications, it is desirable to preferentially enrich mRNA with respect to other cellular RNAs, such as transfer RNA (tRNA) and ribosomal RNA (rRNA). Most mRNAs contain a poly(A) tail at their 3' end. This allows them to be enriched by affinity chromatography, for example, using oligo(dT) or poly(U) coupled to a solid support, such as cellulose or ~~Sephadex™~~ SEPHADEX® (see Ausubel et al., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, vol. 2, Current Protocols Publishing, New York (1994)). Once bound, poly(A)+ mRNA is eluted from the affinity column using 2 mM EDTA/0.1% SDS.

Please amend paragraph [00142] as follows:

The methods of the invention preferably use a control or reference sample, which can be any suitable sample against which changes in cellular constituents can be determined. In one embodiment, the control or reference sample is generated by pooling together the plurality of cellular constituents, e.g., a plurality of transcripts or cDNAs, or a plurality of protein species, from a plurality of breast cancer patients. Alternatively, the control or reference sample can be generated by pooling together purified or synthesized cellular constituents, e.g., a plurality of purified or synthesized transcripts or cDNAs, a plurality of purified or synthesized protein species. In one embodiment, synthetic RNAs for each transcripts or cDNAs are pooled to form the control or reference sample. Preferably, the abundances of synthetic RNAs are approximately the abundances of the corresponding transcripts in a real tumor pool. The differential expression of marker genes for each individual patient sample is measured against this control sample. In one embodiment, 60-mer oligonucleotides corresponding to the probe sequences on a microarray used to assay the expression levels of the diagnostic/prognostic

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS PLLC
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

transcripts are synthesized and cloned into ~~pBluescript~~ PBLUESCRIPT® SK- vector (~~Statagene~~ Stratagene, La Jolla, CA), adjacent to the T7 promotor sequence. Individual clones are isolated, and the sequences of their inserts are verified by DNA sequencing. To generate synthetic RNAs, clones are linearized with EcoRI and a T7 in vitro transcription (IVT) reaction is performed by ~~MegaScript~~ MEGASCRIPT® kit (Ambion, Austin, TX), followed by DNase treatment of the product. Synthetic RNAs are purified on ~~RNeasy~~ RNEASY® columns (Qiagen, Valencia, CA). These synthetic RNAs are transcribed, amplified, labeled, and mixed together to make the reference pool. The abundance of those synthetic RNAs are chosen to approximate the abundances of the transcripts of the corresponding marker genes in the real tumor pool.

Please amend paragraph [00187] as follows:

The polynucleotide molecules which may be analyzed by the present invention (the "target polynucleotide molecules") may be from any clinically relevant source, but are expressed RNA or a nucleic acid derived therefrom (*e.g.*, cDNA or amplified RNA derived from cDNA that incorporates an RNA polymerase promoter), including naturally occurring nucleic acid molecules, as well as synthetic nucleic acid molecules. In one embodiment, the target polynucleotide molecules comprise RNA, including, but by no means limited to, total cellular RNA, poly(A)$^+$ messenger RNA (mRNA) or fraction thereof, cytoplasmic mRNA, or RNA transcribed from cDNA (*i.e.*, cRNA; see, *e.g.*, Linsley & Schelter, U.S. Patent Application No. 09/411,074, filed October 4, 1999, or U.S. Patent Nos. 5,545,522, 5,891,636, or 5,716,785). Methods for preparing total and poly(A)$^+$ RNA are well known in the art, and are described generally, *e.g.*, in Sambrook *et al.*, MOLECULAR CLONING - A LABORATORY MANUAL (2ND ED.), Vols. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1989). In one embodiment, RNA is extracted from cells of the various types of interest in this invention using

guanidinium thiocyanate lysis followed by CsCl centrifugation (Chirgwin *et al.*, 1979, *Biochemistry* 18:5294-5299). In another embodiment, total RNA is extracted using a silica gel-based column, commercially available examples of which include ~~RNeasy~~ RNEASY® (Qiagen, Valencia, California) and ~~StrataPrep~~ STRATAPREP® (Stratagene, La Jolla, California). In an alternative embodiment, which is preferred for *S. cerevisiae*, RNA is extracted from cells using phenol and chloroform, as described in Ausubel *et al.*, eds., 1989, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Vol. III, Green Publishing Associates, Inc., John Wiley & Sons, Inc., New York, at pp. 13.12.1-13.12.5). Poly(A)$^+$ RNA can be selected, *e.g.*, by selection with oligo-dT cellulose or, alternatively, by oligo-dT primed reverse transcription of total cellular RNA. In one embodiment, RNA can be fragmented by methods known in the art, *e.g.*, by incubation with $ZnCl_2$, to generate fragments of RNA. In another embodiment, the polynucleotide molecules analyzed by the invention comprise cDNA, or PCR products of amplified RNA or cDNA.

Please amend paragraph [00190] as follows:

In a preferred embodiment, the detectable label is a luminescent label. For example, fluorescent labels, bioluminescent labels, chemiluminescent labels, and colorimetric labels may be used in the present invention. In a highly preferred embodiment, the label is a fluorescent label, such as a fluorescein, a phosphor, a rhodamine, or a polymethine dye derivative. Examples of commercially available fluorescent labels include, for example, fluorescent phosphoramidites such as ~~FluorePrime~~ FLUOREPRIME® (Amersham Pharmacia, Piscataway, N.J.), ~~Fluoredite~~ FLUOREDITE™ (Millipore, Bedford, Mass.), [[FAM]] FAM™ (ABI, Foster City, Calif.), and [[Cy3]] CY3™ or [[Cy5]] CY5™ (Amersham Pharmacia, Piscataway, N.J.). In another embodiment, the detectable label is a radiolabeled nucleotide.

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS PLLC
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

Please amend paragraph [00197] as follows:

Signals are recorded and, in a preferred embodiment, analyzed by computer, *e.g.*, using a 12 or 16 bit analog to digital board. In one embodiment the scanned image is despeckled using a graphics program (*e.g.*, ~~Hijaak~~ HIJAAK® Graphics Suite) and then analyzed using an image gridding program that creates a spreadsheet of the average hybridization at each wavelength at each site. If necessary, an experimentally determined correction for "cross talk" (or overlap) between the channels for the two fluors may be made. For any particular hybridization site on the transcript array, a ratio of the emission of the two fluorophores can be calculated. The ratio is independent of the absolute expression level of the cognate gene, but is useful for genes whose expression is significantly modulated in association with the different breast cancer-related condition.

Please amend paragraph [00206] as follows:

COMPUTER-FACILITATED ANALYSIS. The analytic methods described in the previous sections can be implemented by use of the following computer systems and according to the following programs and methods. A computer system comprises internal components linked to external components. The internal components of a typical computer system include a processor element interconnected with a main memory. For example, the computer system can be based on an Intel 8086-, 80386-, 80486-, ~~Pentium™~~ PENTIUM®, or ~~Pentium™~~ PENTIUM®-based processor with preferably 32 MB or more of main memory. The computer system may also be a ~~Macintosh~~ MACINTOSH® or a ~~Macintosh~~ MACINTOSH®-based system, but may also be a minicomputer or mainframe.

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS™PLLC
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

Please amend paragraph [00209] as follows:

Loaded into memory during operation of this system are several software components, which are both standard in the art and special to the instant invention. These software components collectively cause the computer system to function according to the methods of this invention. These software components are typically stored on the mass storage device. A software component comprises the operating system, which is responsible for managing computer system and its network interconnections. This operating system can be, for example, of the ~~Microsoft Windows®~~ MICROSOFT® WINDOWS® family, such as ~~Windows~~ WINDOWS® 3.1, ~~Windows~~ WINDOWS® 95, ~~Windows~~ WINDOWS® 98, ~~Windows~~ WINDOWS® 2000, or ~~Windows NT~~ WINDOWS NT®, or may be of the ~~Macintosh~~ MACINTOSH® OS family, or may be UNIX, a UNIX derivative such as LINUX, or an operating system specific to a minicomputer or mainframe. The software component represents common languages and functions conveniently present on this system to assist programs implementing the methods specific to this invention. Many high or low level computer languages can be used to program the analytic methods of this invention. Instructions can be interpreted during run-time or compiled. Preferred languages include C/C++, FORTRAN and JAVA. Most preferably, the methods of this invention are programmed in mathematical software packages that allow symbolic entry of equations and high-level specification of processing, including some or all of the algorithms to be used, thereby freeing a user of the need to procedurally program individual equations or algorithms. Such packages include ~~Mathlab~~ MATLAB® from Mathworks (Natick, MA), ~~Mathematica®~~ MATHEMATICA® from Wolfram Research (Champaign, IL), or ~~S-Plus®~~ S-PLUS® from Math Soft (Cambridge, MA). Specifically, the software component includes the analytic methods of the invention as programmed in a procedural language or symbolic package.

Please amend paragraph [00220] as follows:

Additionally, because the data obtained and analyzed in the software and computer system products of the invention may be confidential, the software and/or computer system preferably comprises access controls or access control routines, such as password protection and preferably, particularly if information is to be transmitted between computers, for example, over the Internet, encryption of the data by a suitable encryption algorithm (*e.g.*, [[PGP]] PGP®).

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS PLLC
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100